

Attorney Docket No.: PTQ-0037
Inventors: Van Eyk et al.
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This listing of the claims will replace all prior versions and listings of claims in the application:

Listing of the claims:

Claim 1: (currently amended) A method of separating a mixture of proteins in a biological fluid sample comprising:

(a) substantially denaturing albumin in said biological fluid sample, wherein mixing the said biological fluid sample is mixed with a solution comprising a sulfhydryl reducing agent, an anionic detergent, and at least one detergent selected from the group consisting of an ionic detergent, a non-ionic detergent and a zwitterionic detergent, at concentrations sufficient to substantially denature albumin in the mixture; and

(b) subjecting the mixture of biological sample and solution to a separation technique to separate proteins in the mixture.

Claim 2: (original) The method of claim 1 further comprising characterizing the separated proteins.

Claim 3: (original) The method of claim 2 wherein the separated proteins are characterized by Western blot.

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Claim 4: (original) The method of claim 1 wherein the biological sample comprises serum.

Claim 5: (original) The method of claim 1 further comprising heating the mixture from step (a) prior to separation in step (b).

Claim 6: (original) The method of claim 5 wherein the mixture is boiled.

Claim 7: (original) The method of claim 1 wherein said separation technique is performed using SDS-PAGE.

Claim 8: (original) The method of claim 1 wherein the anionic detergent is sodium dodecyl sulfate.

Claim 9: (original) A kit for separating a mixture of proteins in a biological sample comprising:

(a) a solution containing a sulphydryl reducing agent, an anionic detergent, and at least one detergent selected from the group consisting of an ionic detergent, a non-ionic detergent and a zwitterionic detergent; and

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(b) instructions for separating proteins in said serum.

Claim 10: (original) A method of assessing cellular injury in a subject comprising:

(a) separating a mixture of proteins in a biological sample of the subject in accordance with the method of claim 1; and

(b) characterizing the separated proteins, wherein said characterization is indicative of cellular injury in the subject.

Claim 11: (original) The method of claim 10 wherein the separated proteins are characterized by Western blot.

Claim 12: (original) The method of claim 10 wherein the characterized protein is at least one of troponin I and troponin T.

Claim 13: (original) The method of claim 10 wherein the cells are cardiac muscle cells.

Claim 14: (original) The method of claim 10 wherein the cells are skeletal muscle cells.

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Claim 15: (original) The method of claim 14 wherein the characterized proteins comprise at least one of a fast and a slow isoform of TnI.

Claim 16: (original) A method of profiling proteins in a biological sample comprising:

- (a) separating proteins of the biological sample in accordance with the method of claim 1; and
- (b) characterizing proteins so as to produce a profile of proteins in said biological sample.

Claim 17: (original) The method of claim 16 wherein the separated proteins are characterized by Western blot.

Claim 18: (original) A method for detecting myocardial damage in a subject comprising detecting a myofilament protein in serum of the subject by Western Blot-Direct Serum Analysis.

Claim 19: (original) The method of claim 18 wherein detection of the myofilament protein in the serum of the patient provides an early clinical assessment or diagnosis of myocardial damage.

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Claim 20: (original) A method for clinically assessing or diagnosing in a subject myocardial damage prior to detection by electrocardiogram or routine clinical testing showing significant elevations of biochemical cardiac markers in the subject, said method comprising detecting by Western Blot-Direct Serum Analysis a myofilament protein in serum of the subject.

Claim 21: (original) A method for monitoring the state of the myocardium in a subject, said method comprising monitoring myofilament protein modifications in serum of the subject by Western Blot-Direct Serum Analysis.

Claim 22: (original) The method of claim 21 wherein monitoring is performed prior to detection by electrocardiogram or routine clinical testing showing significant elevations of biochemical cardiac markers in the subject.

Claim 23: (original) A method for assessing severity of skeletal muscle damage in a subject comprising measuring a ratio of two different isoforms of a myofilament protein in serum of the subject by Western Blot-Direct Serum Analysis.

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Claim 24: (original) The method of claim 23 wherein the two different isoforms of the myofilament protein are fast and slow troponin I or fast and slow troponin T.

Claim 25: (original) A method for diagnosing skeletal muscle damage in a subject comprising measuring a ratio of two different isoforms of a myofilament protein in serum of the subject by Western Blot-Direct Serum Analysis.

Claim 26: (original) The method of claim 25 wherein the two different isoforms of the myofilament protein are fast and slow troponin I or fast and slow troponin T.

Claim 27: (original) A method for differentially diagnosing skeletal muscle damage in a subject comprising measuring a ratio of two different isoforms of a myofilament protein in serum of the subject by Western Blot-Direct Serum Analysis.

Claim 28: (original) The method of claim 27 wherein the two different isoforms of the myofilament protein are fast and slow troponin I or fast and slow troponin T.

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Claim 29: (new) The method of claim 1, wherein the biological sample comprises plasma.

Claim 30: (new) The method of claim 1, wherein the biological sample comprises urine.

Claim 31: (new) The method of claim 1, wherein the biological sample comprises amniotic fluid.

Claim 32: (new) The method of claim 1, wherein the biological sample comprises cerebrospinal fluid.

Claim 33: (new) The method of claim 1, further comprising diluting said mixture of biological sample and solution prior to said separating step.

Claim 34: (new) The method of claim 1, wherein said separation technique is affinity-based.

Claim 35: (new) The method of claim 1, wherein said separation technique comprises chromatography.

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Claim 36: (new) The method of claim 35, wherein said chromatography is high performance liquid chromatography (HPLC).

Claim 37: (new) The method of claim 1, wherein at least one protein of said mixture of proteins is contacted with an antibody thereto.

Claim 38: (new) The method of claim 1, wherein at least one protein of said mixture is contacted with a first antibody thereto, and said first antibody is contacted with a second antibody to the first antibody.

Claim 39: (new) The method of claim 2, wherein said characterizing step identifies at least one protein associated with myocardial damage.

Claim 40: (new) The method of claim 2, wherein said characterizing step identifies at least one protein associated with skeletal muscle damage.

Claim 41: (new) The method of claim 2, wherein said characterizing step comprises fluorescence detection.

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Claim 42: (new) The method of claim 2, wherein said characterizing step comprises colorimetric detection.

Claim 43: (new) The method of claim 2, wherein said characterizing step comprises radiodetection.

Claim 44: (new) The method of claim 2, wherein said characterizing step comprises detection using radiographic film.

Claim 45: (new) The method of claim 2, wherein said characterizing step comprises detecting enzyme activity.

Claim 46: (new) The method of claim 45, wherein said enzyme activity is horseradish peroxidase activity.

Claim 47: (new) The method of claim 45, wherein said enzyme activity is alkaline phosphatase activity.

Claim 48: (new) The method of claim 8, wherein said sodium dodecyl sulfate is present in a concentration of from about 5 mM to about 150 mM.

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Claim 49: (new) The method of claim 1, wherein said zwitterionic detergent is at least one detergent selected CHAPS and a N-alkyl-N,N-dimethylammonio-1-propanesulfonate.

Claim 50: (new) The method of claim 1, wherein said zwitterionic detergent is CHAPS, at a concentration of from about 5 mM to about 50 mM.

Claim 51: (new) The method of claim 1, wherein said non-ionic detergent is at least one detergent selected from Ipegal CA-360, Triton X-100, Triton X-114, n-octyl-glucoside, digitonin, Tween, Tween 20, Tween 80, and saponin.

Claim 52: (new) The method of claim 1, wherein said non-ionic detergent is Ipegal CA-360, in an amount of from about 0.2 % to about 4 %.

Claim 53: (new) The method of claim 1, wherein said sulfhydryl reducing agent is at least one agent selected from dithiothreitol, dithioerythritol, and β -mercaptoethanol.

Claim 54: (new) The method of claim 1, wherein said

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sulfhydryl reducing agent is dithiothreitol, at a concentration of from about 5 mM to about 150 mM.

Claim 55: (new) The method of claim 1, wherein said solution further comprises urea.

Claim 56: (new) The method of claim 55, wherein said urea is present at a concentration of from about 0.2 M to about 4 M.